IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Nobuaki TAKAHASHI *et al.*

Title: **ANTI-CD40 ANTIBODY MUTANTS**

Appl. No.: 10/584,345

Examiner: Phillip GAMBEL

Art Unit: 1644

Confirmation Number: 3671

DECLARATION OF DR. NOBUAKI TAKAHASHI UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Dr. Nobuaki Takahashi, hereby declare and state as follows:

1. I currently hold the position of Senior Scientist in the Antibody Research Laboratories Research Division of KYOWA HAKKO KIRIN CO., LTD. ("KHK"), Japan, the assignee of the captioned application.

2. I have been employed by KHK since 1995, when I obtained my Ph.D. from Osaka University. I worked at Gemini Science, Inc., a subsidiary of KHK in the U.S., from 1998 to 2001. In that position I generated and evaluated various monoclonal antibodies. Since that time I have worked on the research and development of monoclonal antibodies.

3. I am a co-inventor of the captioned application, U.S. Appl. No. 10/584,345 entitled ANTI-CD40 ANTIBODY MUTANTS. It is expected that I would be remunerated when KHK gains income from commercializing a product or technology that is related to the invention of the present application.

4. I have read the U.S. Patent & Trademark Office's Office Action dated October 6, 2009, and publications cited therein: International Patent Application Publication No. WO 02/088186 to Mikayama *et al.*, ("Mikayama PCT"); U.S. Patent No. 7,193,064 to Mikayama *et al.* ("Mikayama"); U.S. Patent No. 6,998,124 to Erickson-Miller *et al.* ("Erickson-Miller"); U.S. Patent No. 6,936,698 to Taylor ("Taylor"); U.S. Patent No. 6,376,653 to Holmes *et al.* ("Holmes").

5. I am co-inventor of the subject matter contained in the Mikayama PCT (WO 02/088186) and Mikayama (USPN 7,193,064). Both Mikayama and the Mikayama PCT describe antibodies which recognize and bind CD40, a molecule present on the surface of B cells and dendritic cells that plays a role in the immune system, including humoral immunity. Mikayama and the Mikayama PCT describe a specific anti-CD40 antagonistic antibody designated "4D11."

6. I understand that the subject matter of the captioned application, U.S. Appl. No. 10/584,345, is directed to an antibody designated "4D11G4PE;" compositions which comprise the 4D11G4PE antibody; and a method of treating or preventing transplant rejection which comprises administering the 4D11G4PE antibody to a patient.

7. The 4D11G4PE antibody is a mutant of the 4D11 antibody and is also an anti-CD40 antagonistic antibody. The 4D11G4PE antibody contains the variable regions of the heavy and light chains of the 4D11 antibody (disclosed in the Mikayama PCT as SEQ ID NOs 48 and 50, respectively). Additionally, the 4D11G4PE antibody contains the constant region of the human IgG4 subclass of antibodies with two amino acid mutations in the constant region of the heavy chain. Specifically, the 4D11G4PE antibody contains two amino acid substitutions at positions S228P and L235E, as indicated by the EU index of Kabat *et al.*, a standard antibody notation system. "S228P" and "L235E" indicate that the serine amino acid at Kabat position 228 was substituted for the amino acid proline and that the leucine amino acid at Kabat position 235 was substituted for the amino acid glutamic acid. The mature heavy and light chains of the 4D11G4PE antibody in the captioned application correspond to amino acids 27 to 474 of SEQ ID NO: 140 and amino acids 23 to 235 of SEQ ID NO: 142, respectively.

8. Examiner Gambel believes, I understand, that a person such as myself would have been motivated to produce the 4D11G4PE antibody based on the disclosure of the 4D11 antibody in the Mikayama PCT and Mikayama in combination with the disclosure of antibodies which recognize other targets but contain the S228P and L235E mutations in Erickson-Miller, Taylor and Holmes. I disagree with this conclusion for the reasons stated below.

9. An antagonistic anti-CD40 antibody, such as 4D11G4PE, binds to CD40 on B cells and dendritic cells, respectively, to block the interaction between CD40 and CD40L on CD4+ T cells, which in turn inhibits the induction of cellular immunity and humoral immunity. Such an antibody is expected to be useful as a suppressor of rejection, following organ transplantation, or as a drug for treating autoimmune disease.

10. When an antibody that recognizes antigens on cells involved in immune responses, such as B cells and dendritic cells, binds to an antigen on such cells, the Fc region of the antibody interacts with Fc receptors presented by other cells, which induces signals for an immune response in some cases. Therefore, when an antibody such as an antagonistic anti-CD40 antibody is used to suppress rejection after organ transplantation or to treat autoimmune disease, "it is important that anti-CD40 antagonistic antibodies have no activity to induce signals by their *in vivo* crosslinking via Fc receptors, even if the ADCC activity

cannot be detected." U.S. Appl. No. 10/584,345 at page 27, last full sentence. This is so because, if such antibodies "induce an agonistic activity due to some effect after they are administered to patients, however weak [the activity] may be, [then] the symptoms may worsen in contrast to the desired therapeutic effect." U.S. Appl. No. 10/584,345 at page 28 (second full sentence).

11. Accordingly, for an antagonistic anti-CD40 antibody, it is very important to avoid the induction of CD40 signals (*i.e.*, agonistic activity) brought about by the crosslinking of the antibody via Fc receptors *in vivo*. Recognizing this goal but uncertain of its attainment, the inventors of the present invention, including myself, set about producing mutants of the 4D11 antagonistic anti-CD40 antibody. Eventually, the claimed antibody, 4D11G4PE, was discovered that exhibits agonistic activity neither *in vitro* nor *in vivo*.

12. As demonstrated in Example 15 and Figure 16 of the captioned application, the 4D11G4PE antibody does not enhance the production of IL-12, an indicator of agonistic activity *in vivo* as a result of the activation of CD-40 expressing cells, *e.g.*, monocytes, when an anti-CD40 antibody binds such cells. As described above, the lack of agonistic activity *in vivo* is critical to successful use of an anti-CD40 antibody as a suppressor of rejection following organ transplantation or as a therapy for autoimmune disease.

13. Additionally, the 4D11G4PE antibody significantly delayed skin graft rejection in human CD40 transgenic mice, compared to a control group. *See* U.S. Appl. No. 10/584,345 at Example 19 and Figure 22.

14. My understanding is that Erickson-Miller discloses antibodies against the erythropoietin receptor which contain the same S228P and L235E mutations as the 4D11G4PE antibody. Erickson-Miller does not disclose or demonstrate how the S228P and L235E mutations of the heavy chain constant region of an IgG4 antibody effect the agonistic activity of the antibody *in vitro* and *in vivo* due to crosslinking of the antibody to an Fc receptor.

15. Additionally, I understand that Holmes discloses anti-Tie2 receptor antibodies which contain the same S228P and L235E mutations as the 4D11G4PE antibody. Holmes does not disclose or demonstrate how the S228P and L235E mutations of the heavy chain constant region of an IgG4 antibody effect the agonistic activity of the antibody *in vitro* and *in vivo* due to crosslinking of the antibody to an Fc receptor.

16. I also understand that Taylor discloses a monoclonal antibody with reduced immunogenicity which contain the same S228P and L235E mutations as the 4D11G4PE antibody. Taylor does not disclose or demonstrate how the S228P and L235E mutations of the heavy chain constant region of an IgG4 antibody effect the agonistic activity of the antibody *in vitro* and *in vivo* due to crosslinking of the antibody to an Fc receptor.

17. It is my opinion, therefore, that a knowledgeable person, informed by the disclosures of Erickson-Miller, Holmes and Taylor, could not have reasonably predicted whether combining the human IgG4 constant region, with the S228P and L235E mutations

and the variable region from the 4D11 antibody would yield an anti-CD40 antibody that exhibited antagonistic activity *in vitro* and, more importantly, *in vivo*.

18. Indeed, an article by Reddy *et al.*, discloses kelisimab (IgG1) and clenoliximab (IgG4PE), two mutated versions of an anti-CD4 antibody. *See* Reddy *et al.*, *J. Immunology* 164:1925-33 (2000) (submitted herewith). Clenoliximab incorporates the heavy chain constant region of IgG4 and includes the S228P and L235E substitutions, which correspond to the 4D11G4PE antibody. Reddy *et al.* teaches that clenoliximab loses the binding activity to Fc receptor *in vitro* (Figure 4B), which reduces the induction of CD4 modulation *in vitro* (Figure 8A). However, Reddy *et al.* also teaches that clenoliximab induces strong CD4 modulation *in vivo* due to the crosslinking of the antibody on CD4 via Fc receptors. Reddy *et al.* at page 1932, left column ("clenoliximab causes strong CD4 receptor modulation in animal models and in the clinic....") This type of activity *in vivo* must be avoided for an antagonistic anti-CD40 antibody, such as 4D11G4PE, to serve as an effective therapeutic.

19. Accordingly, it is my opinion that the *in vitro* activity of the S228P and L235E substitutions described in Erickson-Miller, Holmes and Taylor provide little guidance on whether an antibody will have similar properties *in vivo*. Antibodies previously described, containing the S228P and L235E substitutions of the 4D11G4PE antibody, displayed different activity *in vivo (e.g.,* clenoliximab*)*. Thus, it was both surprising and unpredictable that the 4D11G4PE antibody would maintain the same activity (*i.e.* antagonistic) *in vitro* as well as *in vivo*. It is my understanding that the unexpected properties of the claimed antibody thus distinguish it over the relevant literature cited by Examiner Gambel.

20. So remarkable are the properties of the claimed antibody that it now is under evaluation in a clinical trial designed to evaluate its suitability as a drug.

21. I declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date _____ By _____

Dr. Nobuaki Takahashi